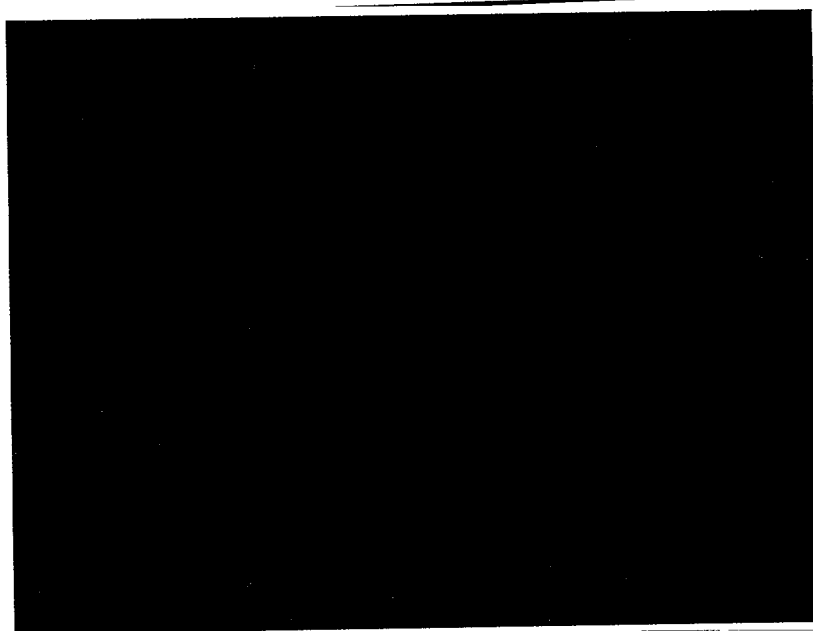


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**GFP-LCA (wt)**

Figure 1

**GFP-TLCA**

Figure 2

1	PEVVKQFENYK	DPVNGVDIAY	IKIPNVGQMQ	PVKAFKIHNK	IWVIPERDTF
51	TNPEEGDLNP	PPEAKQVPVS	YDSTYLSTD	NEKDNYLKGV	TKLFERIYST
101	DLGRMLLTSI	VRGIPFWGGS	TIDTELKVID	TNCINVIQPD	GSYRSEELNL
151	VIIGPSADII	QFECKSFGHE	VLNLTRNGYG	STQYIRFSPD	FTFGFEESLE
201	VDTNPLLGAG	KFATDPAVTL	AHELIHAGHR	LYGIAINPNR	VFKVNTNAYY
251	EMSGLEVSFE	ELRTFGGHDA	KFIDSLQENE	FRLYY <sup>Y</sup> NKFK	DIAS <sup>Y</sup> TLNKAK
301	SIVGTTASLQ	YMKNVFKEY	LLSEDTSGKF	SVDKLKFDKL	YKMLTEIYTE
351	DNFVKFFKVL	NRKTYLNF <sup>*</sup> DK	AVFKINIVPK	VNYTIYDGFN	LRNTNLAANF
401	NGQNT <sup>*</sup> EINNM	NFTKLKNFTG	LFEFYKLLCV	RGII <sup>*</sup> TSK	

Figure 3

## GFP-LCA (AA mut)

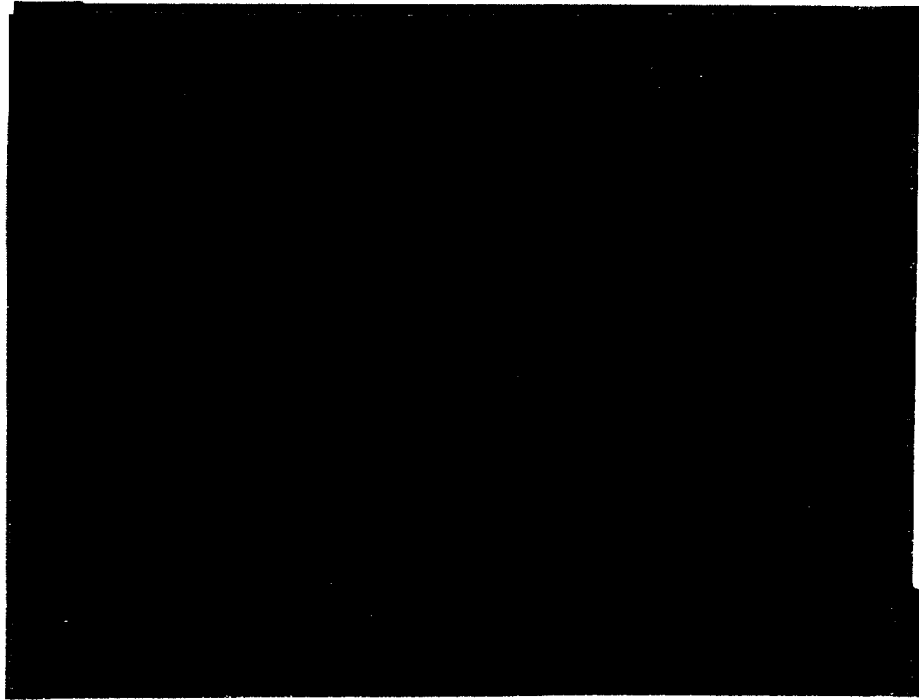


Figure 4

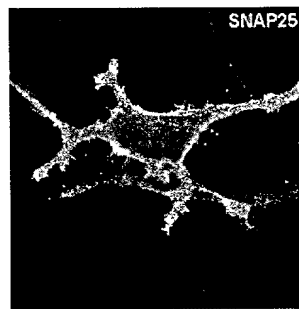
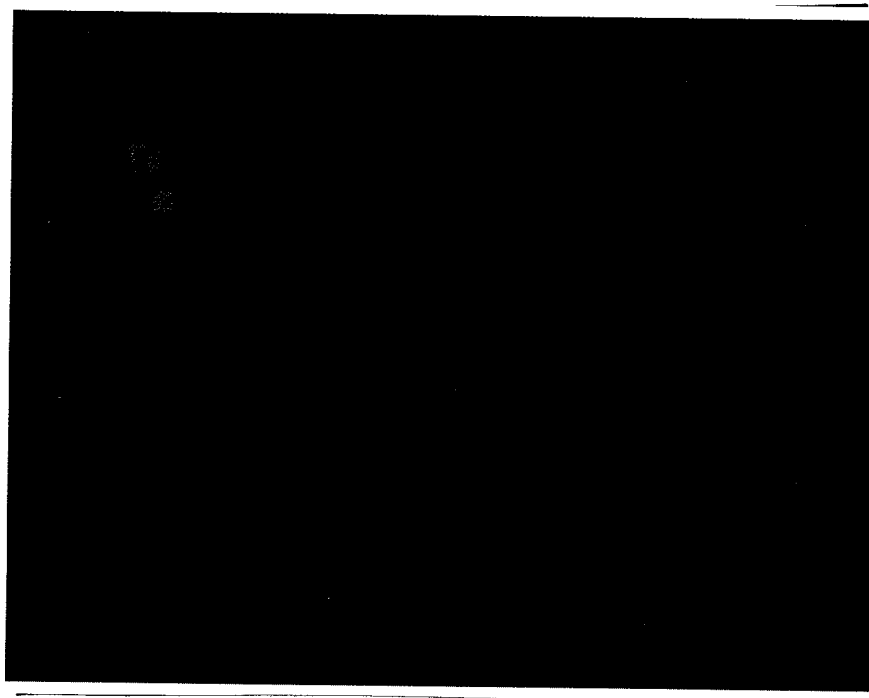


Figure 5





**GFP-LCB(wt)**

Figure 7

BoNT-A_Halla_LC	1	75	1	75
	(1)			
	(1)			
	(1)			
BoNT-B_Danish_I_LC	1	75	1	75
	(1)			
	(1)			
	(1)			
BoNT-A_Halla_LC	76	150	76	150
	(74)			
	(76)			
	(76)			
BoNT-B_Danish_I_LC	76	150	76	150
	(74)			
	(76)			
	(76)			
BoNT-A_Halla_LC	151	225	151	225
	(148)			
	(151)			
	(151)			
BoNT-B_Danish_I_LC	151	225	151	225
	(148)			
	(151)			
	(151)			
BoNT-A_Halla_LC	226	300	226	300
	(218)			
	(226)			
	(226)			
BoNT-B_Danish_I_LC	226	300	226	300
	(218)			
	(226)			
	(226)			
BoNT-A_Halla_LC	301	375	301	375
	(293)			
	(300)			
	(301)			
BoNT-B_Danish_I_LC	301	375	301	375
	(293)			
	(300)			
	(301)			
BoNT-A_Halla_LC	376	447	376	447
	(367)			
	(375)			
	(376)			

Figure 8

# BirA-SNAP25 Assay of rLC/A Activity

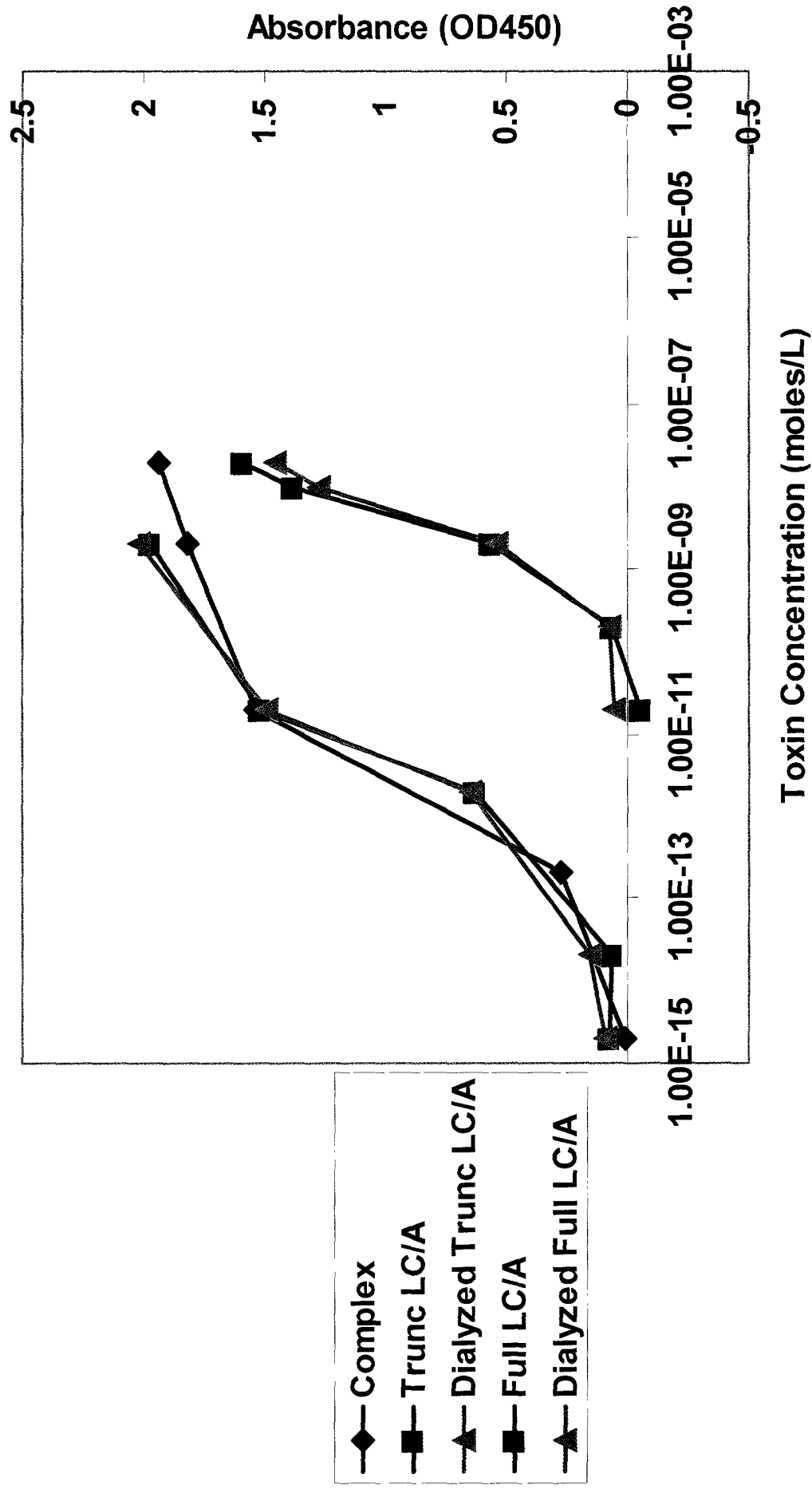


Figure 9



# Comparison of LC/A constructs expressed from E. coli for in vitro analysis

	<u>6xHis</u>	<u>S-tag</u>	
Wildtype LC/A			PFVVKQFNYKDPV...
trun LC/A	MHHHHHSSGLVPRGSGMKE	TAAAKFERQHMDSPDLGTDDDDKAM	.....YKDPV...
N-His LC/A	MHHHHHSSGLVPRGSGMKE	TAAAKFERQHMDSPDLGTDDDDKAMGSFVVKQFNYKDPV...	
Wildtype LC/A	...NFTKLKNFTGL	<b>EFYKLL</b> CVRGIITSK	
trun LC/A	...NFTKL.....	TRAHHHHH	
N-His LC/A	...NFTKLKNFTGL	<b>EFYKLL</b> CVRGIITSK	
		<u>6xHis</u>	

- Truncated LC construct published
  - Kadkhodayan, S. et al. Prot. Exp. Purif. 2000, 19, 125-130
  - Crystal structure reported at IBRCC in Oct. 2000

Figure 10